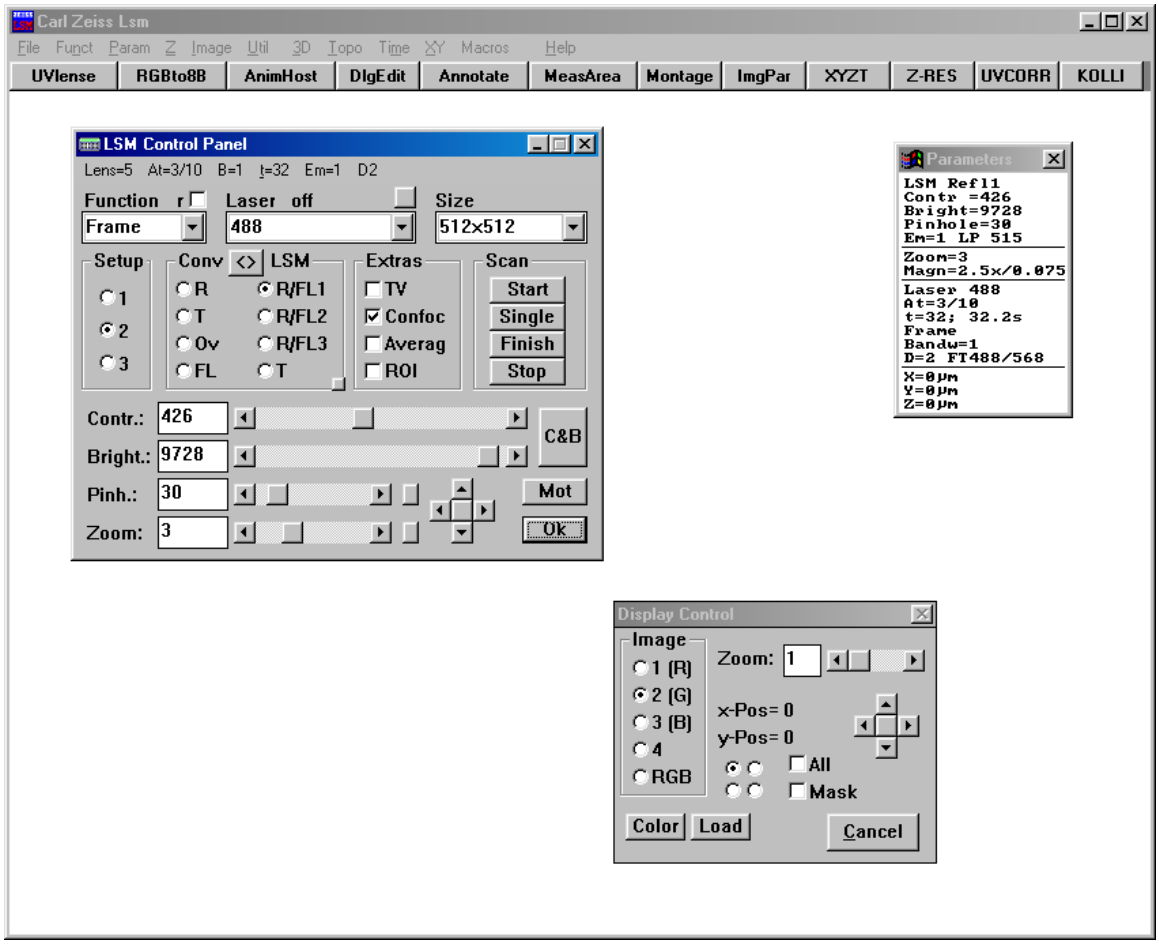
LSM 410

QuickStart Manual

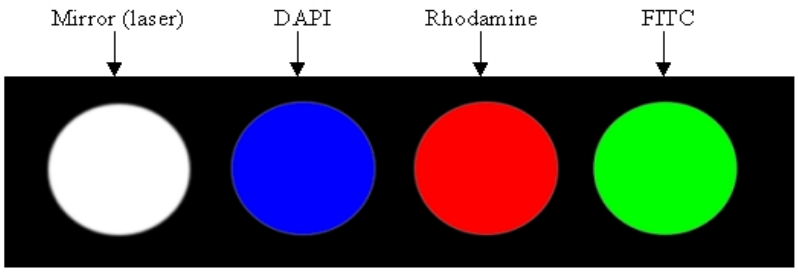
QuickStart Manual for LSM 410

1. Turn on Xenon Arc Lamp.
2. Turn on AC Power Switch on Ar/Kr Laser power supply.
3. Turn key to "**ON**", then hold to "**Start**" for approximately ½ sec and release.
4. Wait Approx. 30 sec until laser fires.
5. Turn on computer (Yellow Key labeled "**Computer Switch**")
6. Software will boot into Windows 98, double click on **LSM** icon.

****Make sure the transmitted light arm of the microscope is in the upright position, and the filter block is in the mirror position (all the way to the right). The 410 system has an alarm system that warns you if you are **NOT** in these positions upon startup)****



The LSM 410 Axiovert 100 has a 4-position filter block (3 viewing, 1 laser) with the following filters:

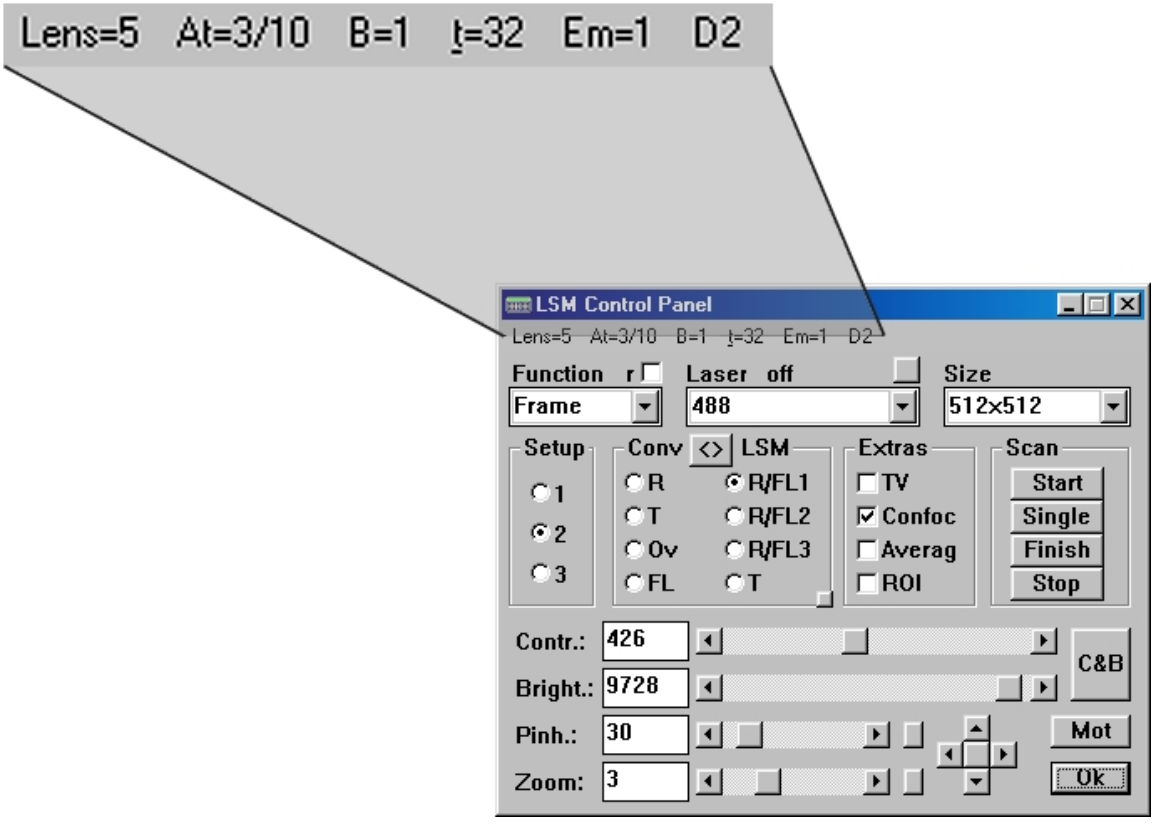


7. Move the filter block from the mirror position to one of the 3 viewing positions.
8. Mount your sample, focus, and center your specimen in the eyepieces as explained in training.
9. Move the filter block from one of its 3 viewing positions (DAPI, Rhodamine, FITC) to the Mirror position.

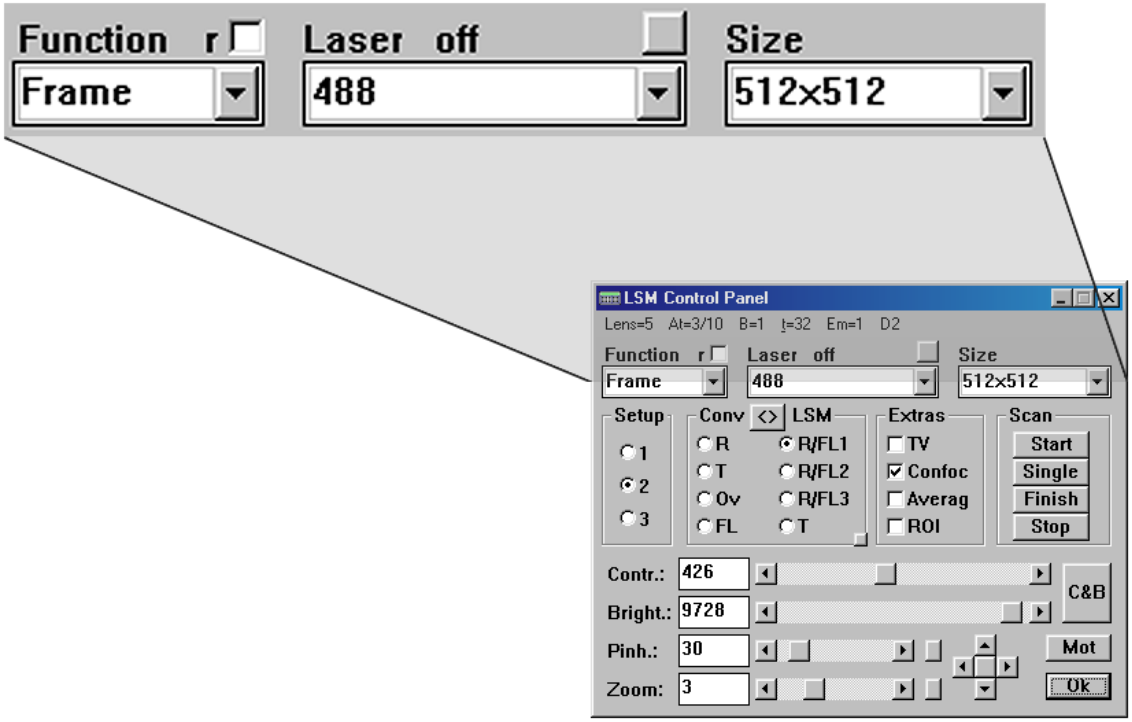
****Make sure all sliders/light blocks are out of the light path at this time****

Control Panel

1. **Lens** = select the appropriate lens (important in determining measurement, pixel dimensions, and resolution information).
2. **At = Laser Attenuation** is the laser intensity control (adds neutral density filters to the light path – the higher the number, the more ND filters in the path ie the lower the amount of light).

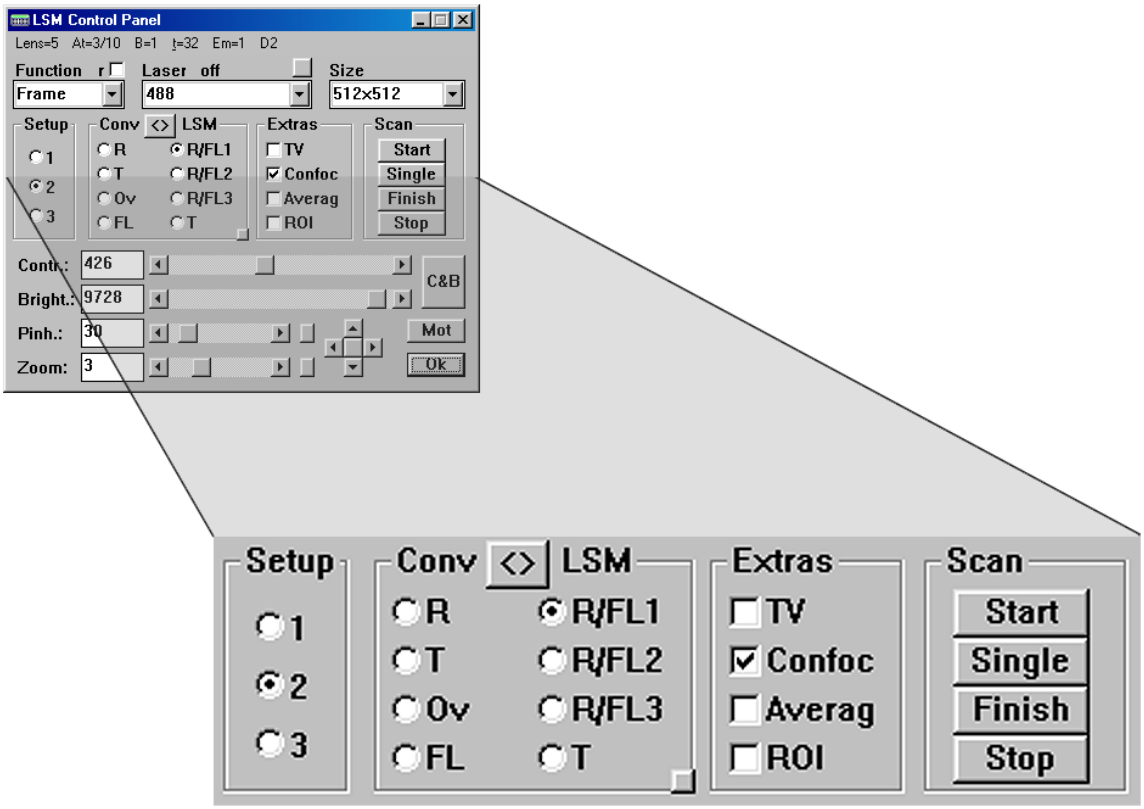


3. **B = Bandwidth Filter** used to improve signal to noise ratio electronically. Most use 1.
4. **t = Time** length of time to scan a 512X512 image. The longer the time, the greater the pixel dwell time, the better the image quality. *Use a 1 second scan to position sample and find focus, use 16 or 32 second scan for final image.*
5. **Em = Emission Filter** for a particular PMT. *Depends on probe*.
6. **D = Primary Dichroic Mirror** or beamsplitter, reflects appropriate laser line to sample, passes emission wavelengths (slightly higher than excitation).



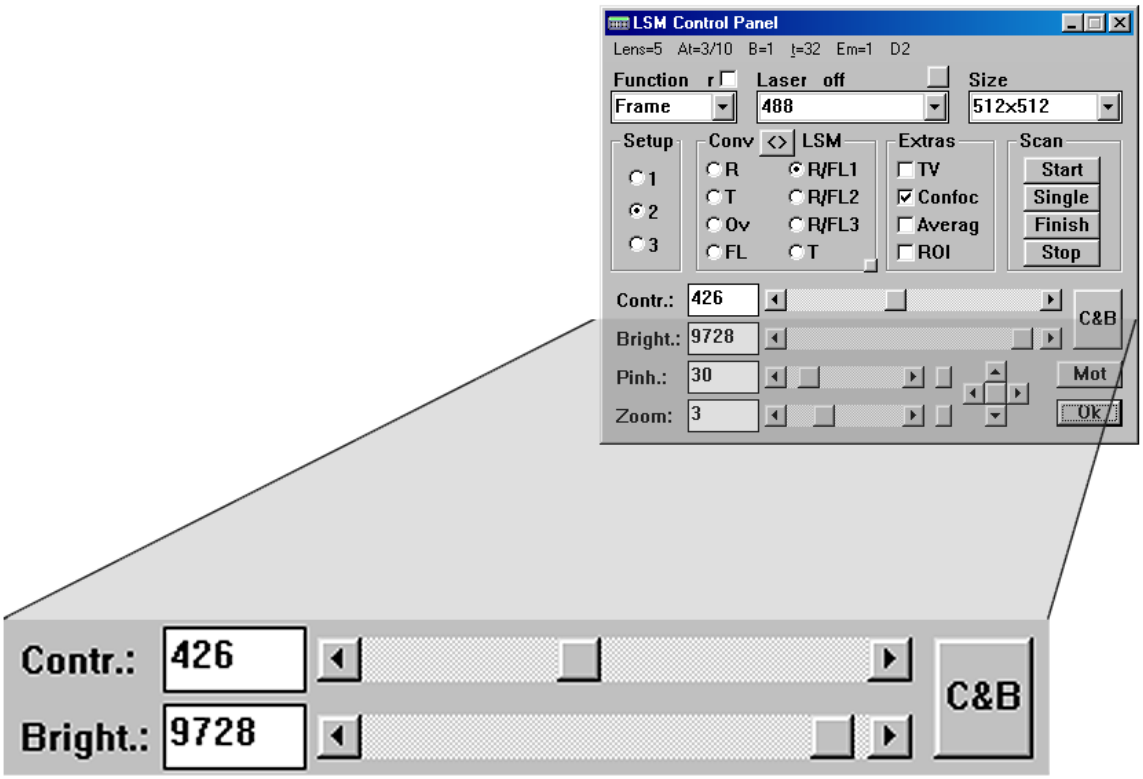
7. **Function** = type of scanning:
   1. **Overl2** - simultaneous acquisition of up to 2 probes.
   2. **Frame** - independent acquisition of up to 3 probes.

8. **Laser =** Excitation laser line. *Depends on probe.*
9. **Size =** digital image size. Remains the same (512X512).



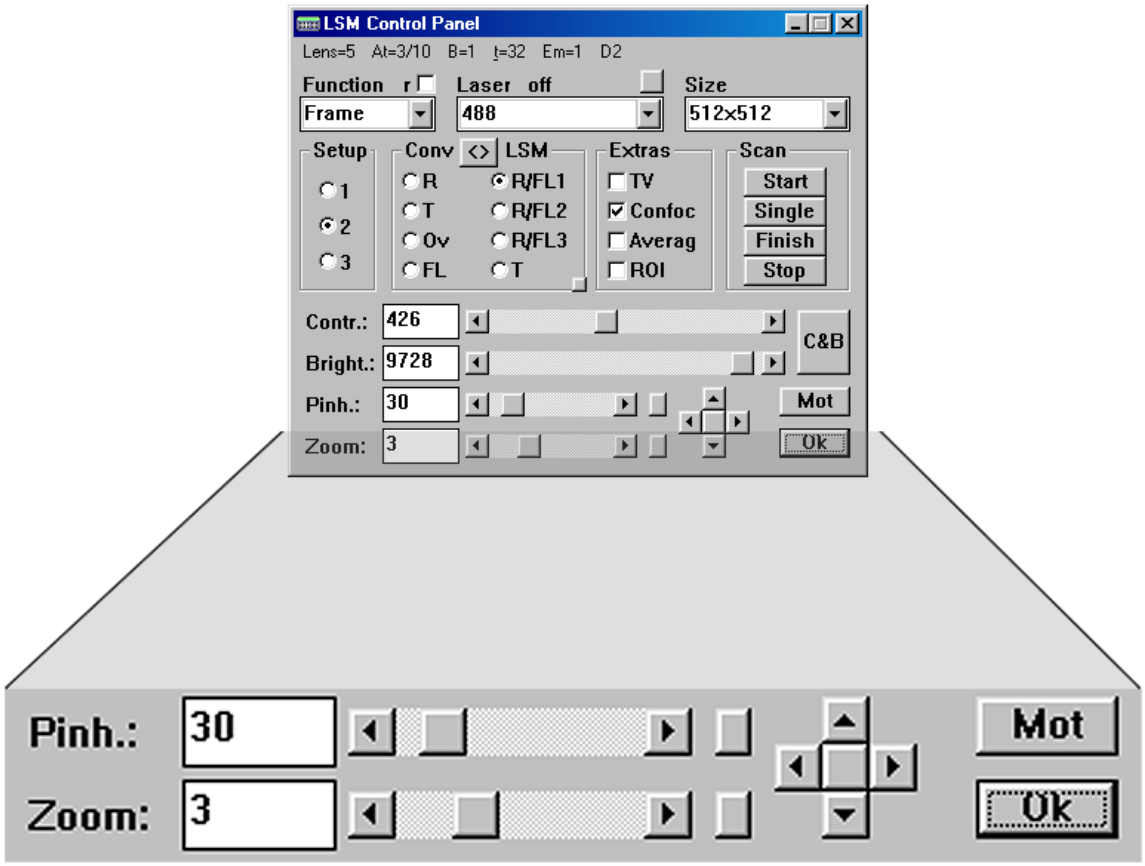
Configuration Setup

1. **Setup** – based on the function you chose, setup will have either 3 (Frame) or 2 (Overl2) selections. Each selection allows you to configure each motorized function in the "**Control Panel**" and the color settings in the "**Display Control**". Design 1 setup for each probe. (Up to 3 probes).
2. **Conv/LSM –** The software switch between using the confocal (LSM) and the eyepieces (Conv). *On the Conv side, T = transmitted light, FL = fluorescence. On the LSM side, R/FL1 = PMT 1, R/FL2 = PMT 2. This system does not have a third detector. (NO R/FL3).*
3. **Extras – Confoc** = when selected, confocal aperture is in place, when deselected the system is effectively widefield. **Averag** = frame averaging tool. **ROI** = region of interest tool.
4. **Scan –** Start initates a continuous scan, Stop terminates the scan. Single makes a single pass over the 512X512 image, and Finish completes a continuous scan.



Controlling How Your Image Looks

1. **Contr.** = gain control on PMT. Controls how sensitive the detector is to a photon of light. The higher the number, the more current passes across the PMT, and more of your signal is amplified. **Bright** = Black level control. Controls the level of your background. As you decrease this number, your background decreases (as well as your signal).
   ****F9 – auto contrast/brightness balance = a good starting point for balancing images****
2. **PinH**. = Pinhole Adjustment (Confocal Aperture). The pinhole is used to improve resolution by removing light outside the focal plane. *A good starting point is 30. Increase the pinhole size if your signal is weak (contrast above 450), and decrease pinhole if signal is strong. Pinhole can only be reduced to 1 airy unit, and the pinhole diameter depends on the objective lens used. These calculations are found in "**ZRES**" macro on toolbar.*
3. **Zoom =** optical zoom tool.



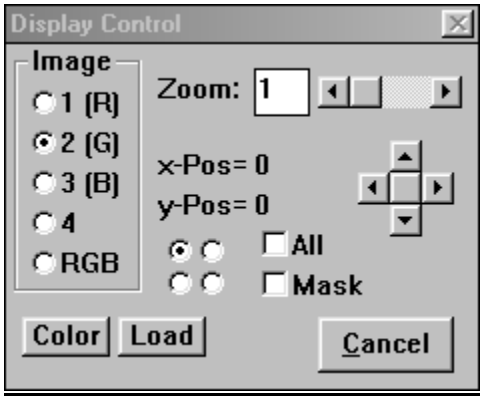
Display Control

The Display Control determines how your collected image is displayed. There are 5 frame stores – red (R), green (G), blue (B), 4 (transmitted light), and RGB (color overlay of images in R, G, and/or B). A grayscale image is generated when selected in either R,G,or B. When RGB is selected, the display is an overlay of any images in the red, green or blue stores.

****Remember, WYSIWYG!! The image displayed is how the image is saved.****

The Color button toggles between 3 look up tables (LUT) within the grayscale image stores (R, G, or B).

1. Grayscale – scale from 0-255 (8 bit)
2. Indexed Color – scale from 0-255 where the 0 level pixels are colored blue, and the 255 level pixels are colored red. This map is useful when quantitation is necessary, and you must keep the dynamic range of your image below saturation.
3. Glowscale – red and yellow map used in materials microscopy. Useless for biological samples.



Saving Images

Once an image is complete and you wish to save:

1. Push "**File**" and "**Store Image**".
2. Navigate to your assigned directory in the subpath C:\Images.
3. Assign a file name (no more than 8 characters).
4. Push "**Store**".

Changing Between Viewing Your Sample Via LSM and Using Eyepieces

1. When viewing through the oculars, click either T or FL under the "**Conv/LSM**".

   ****There is a warning box stating you must move the filter block.****

2. Move the filter block to an appropriate filter set (DAPI, FITC, Rhodamine).
3. Once you have found a specimen, move the filter block back to the mirror position.
4. Click the appropriate PMT (R/FL1, 2) for LSM detection under "**Conv/LSM**".

System Shutdown

1. Quit the LSM software.
2. Shutdown Windows 98 via Start menu.
3. When the screen says it is safe, turn off yellow switch marked "**Computer**".
4. Turn off Ar/Kr laser by turning key counter-clockwise to OFF position.
5. Turn off Xenon Arc Lamp.
6. WAIT 5 minutes, shut off AC power on Laser power supply.